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EVALUATION OF THE EMBRYOTOXICITY OF JP-10 IN THE RAT

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ABSTRACT

The effect of JP-10, the major component of cruise missile fuel, on the development of embryonic rats was evaluated. Pregnant females were exposed via inhalation to 600 ppm or orally dosed with 250, 500 or 1000 mg JP-10/kg on gestation days 6-15. In a separate experiment both fetal and maternal blood levels of JP-10 were monitored during an inhalation exposure. Moderate signs of toxicity including tremors and convulsions were observed in the pregnant females receiving the higher doses. JP-10 was not selectively embryotoxic in the rat when administered by gavage or inhalation. Blood levels of JP-10 in the fetuses were about one half the maternal blood levels at steady state.

INTRODUCTION

JP-10, the exoisomer of 4,7-methano-1-H-indene, octahydro 12aα, 4α, 7α, 7aα, is the major component of cruise missile fuel. It is a synthetic saturated polycyclic hydrocarbon with a molecular structure similar to the cyclodiene chlorinated insecticides, except no chlorine atoms are present (Figure 1). The fuel consists of 98.5% JP-10; the endoisomer is the major impurity. As

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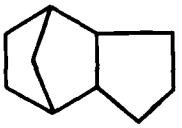

Fig. 1 JP-10 structure

with other fuels, it is expected that JP-10 will occasionally be involved in high level accidental exposures and environmental spills.

JP-10 is not very toxic.[1] In acute inhalation studies, mice were more susceptible than rats and hamsters with an LC_{50} 4 hr slightly greater than 900 ppm. Toxic signs observed in these exposures were eye irritation, tremors, prostration, convulsions, and death. The primary metabolite of JP-10 in the rat is 5-hydroxy exo-JP-10.[2] In a previous study no evidence of embryotoxicity was found when pregnant mice were treated orally with JP-10 during the period of organogenesis.[3] The purpose of this study was to evaluate the embryotoxic potential of JP-10 in Fischer 344 rats following oral or inhalation exposure to a maximum tolerated dose or concentration.

MATERIALS AND METHODS

Animals

Virgin female Fischer 344 rats (Charles River Breeding Laboratories) were housed in plastic cages containing wood chip bedding in a room maintained at 72-76°F with a 12 hour light cycle. The rats received food (Ralston Purina Co.) and water ad libitum. The females were placed with fertile males of the same stock overnight and checked for presence of sperm by vaginal wash the next morning. The day on which sperm was found was designated day 0 of pregnancy. The pregnant rats were weighed daily. Weights are listed as mean ± standard deviation (S.D.).

Oral JP-10 Exposure

Groups of pregnant rats were dosed with JP-10 (Ashland Oil) by gavage on days 6 through 15 of gestation. Rats received daily doses of 250, 500, or 1000 mg JP-10/kg. The JP-10 was diluted with corn oil and controls received an equivalent volume of corn oil (2.0 ml/kg).

Inhalation JP-10 Exposure

Pregnant rats were exposed to a time weighted average of 600 ppm JP-10 for 6 hours each day on days 6 through 15 of gestation. The rats were exposed in a 66ℓ inhalation chamber with a flow rate of 30ℓ/minute. The chamber JP-10 concentration was monitored every three minutes via a gas sampling valve connected to a gas chromatograph. Controls were held in a JP-10 free inhalation chamber an equivalent time.

Examination of Fetuses

The pregnant females were sacrificed on day 20 of gestation and the fetuses delivered by caesarean section. The number and placement of fetuses and resorption sites were recorded. Fetuses were removed, weighed, sexed, and examined for external abnormalities. About 50 percent of the fetuses in each litter were fixed in Bouin's solution and the remainder in absolute ethanol. Fetuses fixed in Bouin's were serially sectioned with a razor blade and examined for soft tissue abnormalities.[4] Fetuses fixed in ethanol were cleared in KOH, stained with Alizarin Red S and examined for skeletal abnormalities.[5] Measured data were analyzed for statistical significance by the Student's t method and are listed as mean ± standard deviation (S.D.). Incidence data were analyzed with Fisher's exact test. The level of significance chosen for all tests was $p \leq 0.05$. The litter was treated as the experimental unit.

Maternal and Fetal JP-10 Toxicokinetics

A second inhalation exposure was conducted in which pregnant rats were exposed to 600 ppm of JP-10 on gestation day 19. Groups of 3 rats were removed at 15, 30, 60, 120, and 240 minutes to obtain maternal and fetal blood samples for JP-10 analysis. Heparinized blood samples were obtained from the posterior vena cava of the pregnant females. Heparinized blood samples were obtained from the fetuses by decapitation and collection of the blood in small heparinized containers. The blood from individual fetuses was combined to yield a pooled litter blood sample. Analysis of blood for JP-10 was accomplished by extracting a 0.2 ml blood sample with 0.15 ml hexane and injecting 1 μℓ of the hexane extract. The gas chromatograph used was a HP 5880A with a 10 ft 1/8" OD column containing 10 percent SE-30 on chromosorb W-HP 80/100. The column temperature was 150°C, with a programmed increase of 20°/minute to a final temperature of 200°C.

RESULTS

Pregnant rats given JP-10 by gavage or inhalation gained less weight than the controls during the treatment period (Table 1). The decreased weight gain was dose related with both the 1000 mg/kg and 500 mg/kg orally dosed groups and the inhalation treatment group having significantly less weight gain during the early phase of JP-10 treatment. The 1000 mg/kg group and the inhalation treatment group also gained significantly less weight during the latter phase of JP-10 treatment. The values for total weight gained during gestation (days 6-20) and also maternal weight gain (gestation day 20 weight with gravid uterus removed - gestation day 0 weight) were also significantly reduced in the 1000 mg/kg treatment group. The weight gain following cessation of JP-10 treatment was not significantly different from the control for any group (Table 1). In addition to a consistently

TABLE 1

Effect of JP-10 on Weight Gains of Pregnant Rats

	JP-10 Dose[a]					
	mg/kg/day[b]				Inhalation (ppm)[c]	
	0	250	500	1000	600	0
Number of animals	23	21	21	21	17	18
Body wt on day 0[d] (gms)	172 ± 11	175 ± 14	173 ± 8	174 ± 15	186 ± 10	185 ± 12
Wt gain of females during JP-10 treatment (gms) (gestation days)						
6-10[d]	9 ± 6	7 ± 5	1 ± 4[e]	-1 ± 7[e]	2 ± 3[e]	4 ± 3
11-16[d]	21 ± 3	23 ± 3	21 ± 4	16 ± 5[e]	13 ± 7[e]	21 ± 3
17-20[d]	33 ± 8	36 ± 8	37 ± 8	29 ± 12	32 ± 11	28 ± 5
Total (6-20)[d]	62 ± 12	65 ± 13	59 ± 10	45 ± 15[e]	47 ± 15	53 ± 7
Maternal wt gain[d] (gms) (day 20 wt with gravid uterus removed - day 0 wt)	23 ± 6	26 ± 6	23 ± 9	14 ± 13[e]	15 ± 7	13 ± 5

[a] All JP-10 treatments occurred on gestation days 6-15.
[b] Oral dosing of JP-10.
[c] Daily 6 hour inhalation exposure.
[d] Mean ± S.D.
[e] Significantly different from control $p \leq 0.05$.

reduced weight gain for the 1000 mg/kg orally dosed group and the inhalation treatment group, many animals in these groups also exhibited tremors and a few also had mild convulsions. The weight gain of the 250 mg/kg orally dosed group was not different from the control, nor were other signs of toxicity observed.

Treatment with JP-10 had an inconsistent effect on litter parameters such as mean fetal weight, incidence of resorptions and incidence of fetal abnormalities (Table 2). There was a statistically significant increase in litters containing resorptions amounting to 25 percent or more of the implants in the high dose group; however, the incidence of resorptions/litter although elevated was not significant. The 500 mg/kg orally dosed group had a significantly reduced fetal weight while the 1000 mg/kg group did not. The incidence of malformation was not significantly elevated for any treatment group (Table 2).

The blood concentration of JP-10 in pregnant females exposed by inhalation reached a plateau in about 1 hour (Fig. 2). Fetal blood required a longer time period to reach a plateau, at a concentration roughly equivalent to 50 percent of the mean maternal JP-10 blood level.

DISCUSSION

JP-10 treatment by inhalation or gavage was not teratogenic in the Fischer 344 rat. The most common major malformation observed in the 2 higher orally dosed groups and the inhalation group was nanoidism (unusually small fetus). This abnormality did not occur in the oral control or lowest dose group in this study. It did occur in the inhalation control and has occurred in other control litters historically in this laboratory at a rate similar to that observed in the higher JP-10 treatment groups. The second most common major malformation involved the urinary tract development. The malformation included hydro-

TABLE 2

Effect of JP-10 on Litter Parameters

	JP-10 Dose[a]					
	mg/kg/day[b]				Inhalation (ppm)[c]	
	0	250	500	1000	600	0
Number of litters	23	21	21	21	17	18
Implants/litter[d]	10.3 ± 2.8	10.6 ± 2.4	11.0 ± 2.4	10.0 ± 2.7	9.1 ± 3.4	9.5 ± 1.8
Resorptions/litter[d]	0.5 ± 0.7	0.6 ± 0.8	0.5 ± 0.6	1.2 ± 2.4	0.6 ± 0.9	0.4 ± 0.9
Number of litters 25% or more resorbed	0	0	0	4[e]	2	0
Fetal wt (gms)[d,f]	3.2 ± .21	3.1 ± .16	3.0 ± .18[e]	3.1 ± .26	3.1 ± .23	3.1 ± .08
Incidence of Abnormalities						
Litters (fetuses) examined	23(219)	21(210)	21(220)	21(184)	17(144)	18(163)
Litters (fetuses) affected	2(2)	4(4)	3(4)[g]	5(7)[h]	2(2)[i]	4(7)[g]
Major malformations[j]	0	0	3	4	3	3
Minor malformations[j]	2	4	2	3	0	4

[a] All JP-10 treatments occurred on gestation days 6-15.
[b] Oral dosing of JP-10.
[c] Daily 6 hour inhalation exposure.
[d] Mean ± S.D.
[e] Significantly different from control $p \leq 0.05$.
[f] Mean fetal wt was determined by obtaining the mean fetal wt for each litter and finding the mean ± S.D. of the litter means.
[g] Major malformation was nanoid (≤ control mean fetal wt - 5 S.D.).
[h] Major malformations were nanoid (1), and a syndrome involving the urinary system including hydronephrosis, dilated ureter, and agenesis of the bladder which occurred in 3 fetuses from 1 litter.
[i] Major malformations included nanoid (2) and microophthalmia (1).
[j] Minor malformations included delayed ossification of vertebral centrae, hypoplastic ribs, displaced or retained testicle, and mild hydronephrosis.

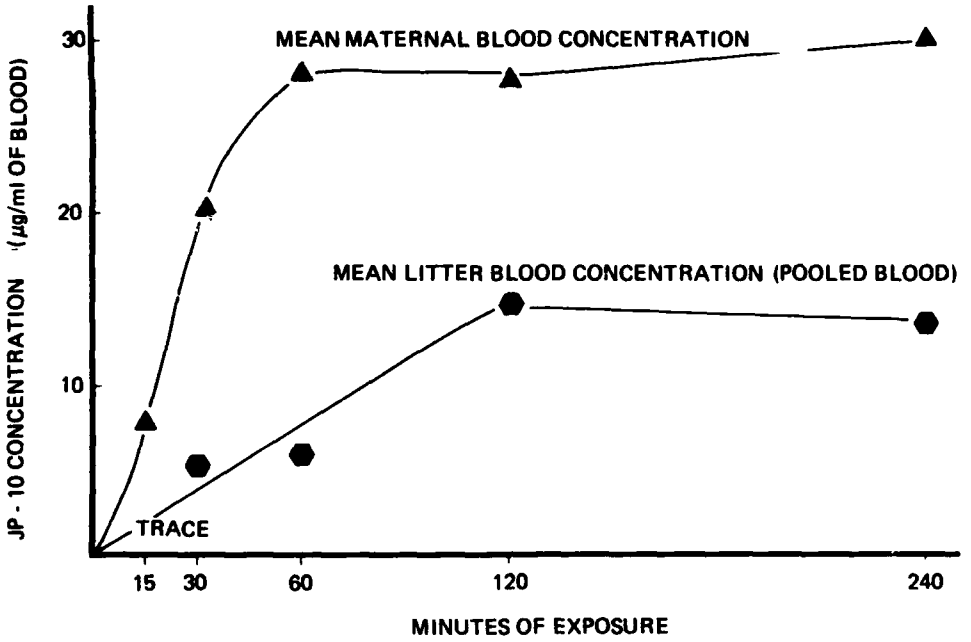

Fig. 2 Maternal and fetal blood JP-10 concentration changes during exposure to 600 ppm JP-10. Each maternal blood data point is the mean of 3 gravid females. Each litter blood data point is the mean of 3 litters. A litter blood sample was obtained by pooling the blood from the individual fetuses of that litter.

nephrosis, dilated ureter, and agenesis of the bladder. It occurred in 3 fetuses from 1 litter. Since this malformation was observed in the fetuses from only one litter it probably arose spontaneously rather than as a result of JP-10 treatment. Thus, the incidence of major malformations at the higher JP-10 doses does not appear significant.

Data concerning nonteratogenic embryotoxicity were ambiguous. There was an increase in embryolethality in the 1000 mg JP-10/kg group and a decrease in fetal weight in the 500 mg JP-10/kg group. These indicators of embryotoxicity were not distributed

in a dose-related manner. Most likely they were secondary to maternal toxicity (reduced weight gains, tremors, and convulsions) in the 500 and 1000 mg JP-10/kg orally dosed groups. The similarity of signs for the 1000 mg JP-10/kg orally dosed group and the 600 ppm inhalation group suggests similar effective doses for these two groups. The 500 mg JP-10/kg orally dosed females were not as severely affected by JP-10 treatment as the higher dose group but weight gains during the early part of gestation were depressed indicating a mild toxic response. The blood level data show fetal blood concentrations to be lower than maternal blood JP-10 concentrations. Thus, JP-10 reaches the fetus but the placenta produces a sparing effect with regard to fetal JP-10 concentration. These blood level data substantiate that a JP-10 exposure that produces moderate toxicity in gravid rats also produces substantial blood levels in the fetus but with negligible embryotoxicity.

In conclusion, JP-10 administered either by gavage or inhalation at sufficient doses to produce moderate maternal toxicity was not selectively embryotoxic.

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